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(54) Title: ANALYTICAL DEVICE
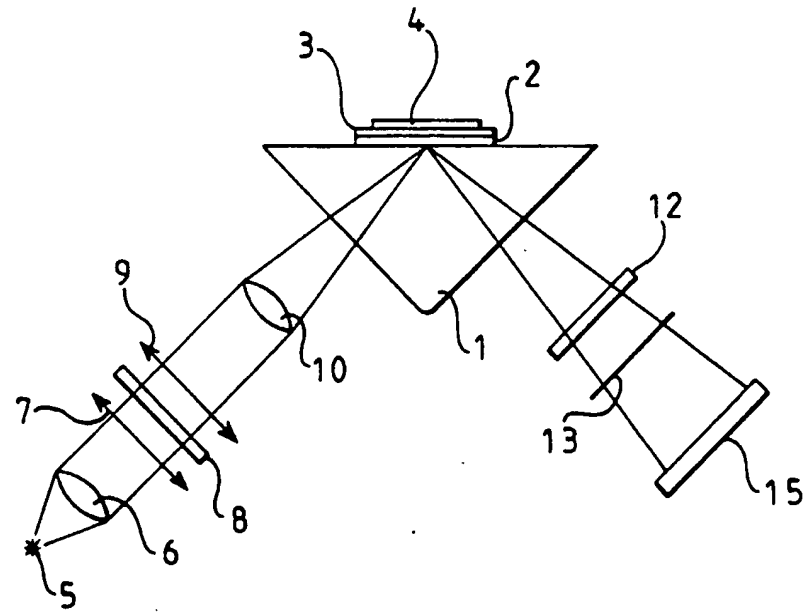
(57) Abstract

Apparatus for the determination of a chemical or biochemical species comprises a resonant optical sensor (1-4) disposed in a light path between a polychromatic light source (5) and a detector (15). The sensor (1-4) is irradiated with light from the light source (5) at a range of angled simultaneously, and light reflected from the sensor (1-4) is allowed to reach the detector (15) only when resonance occurs. The intensity of the polychromatic light from the light source (5) is modulated as a function of frequency, preferably periodically.

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Title : <u>Analytical Device</u>

This invention relates to sensors, especially those termed
biosensors, ie devices for the analysis of biologically active
species such as antigens and antibodies in samples of
biological origin. In particular, the invention relates to
biosensors based on resonant optical phenomena, eg surface
plasmon resonance or resonant attenuated or frustrated total
internal reflection.

Many devices for the automatic determination of biochemical
analytes in solution have been proposed in recent years.
Typically, such devices (biosensors) include a sensitised
coating layer which for optical devices might be located in
the evanescent region of a resonant field. Detection of the
analyte typically utilizes optical techniques such as, for
example, surface plasmon resonance (SPR), and is based on
changes in the thickness and/or refractive index of the
coating layer resulting from interaction of that layer with
the analyte. This causes a change, eg in the angular position
of the resonance.

Other optical biosensors include a waveguide in which a beam
of light is propagated. The optical characteristics of the
device are influenced by changes occurring at the surface of
the waveguide. One form of optical biosensor is based on
frustrated total reflection. The principles of frustrated
total reflection (FTR) are well-known; the technique is
described, for example, by Bosacchi and Oehrle [Applied Optics
(1982), <u>21</u>, 2167-2173]. An FTR device for use in immunoassay
is disclosed in European Patent Application No 2205236A and
comprises a cavity layer bounded on one side by the sample
under investigation and on the other side by a spacer layer
which in turn is mounted on a substrate. The substrate-spacer
layer interface is irradiated with monochromatic radiation
such that total reflection occurs, the associated evanescent
field penetrating through the spacer layer. If the thickness

of the spacer layer is correct and the incident parallel wave
vector matches one of the resonant mode propagation constants,
the total reflection is frustrated and radiation is coupled
into the cavity layer. The cavity layer must be composed of
material which has a higher refractive index than the spacer
layer and which is transparent at the wavelength of the
incident radiation.

SPR could be thought of as a special case of FTR. However,
for present purposes a distinction is drawn between devices in
which energy is coupled into a metal layer (SPR devices) and
devices in which radiation is coupled into a dielectric layer
(FTR devices).

In SPR devices there is a dip in the reflected light intensity
on resonance. Rather than detecting a dark band against a
light background, there are certain advantages in detecting a
bright band against a dark background and this can be achieved
using the method and apparatus disclosed in International
Patent Application WO 92/04617. In devices based on the
principle of FTR, on the other hand, the overall intensity of
the reflected light is substantially unchanged. The light
does, however, undergo a phase change at resonance and, by
appropriate use of polarisers, resonance can be detected as a
bright line on a dark background, or vice versa.

In both SPR and FTR, two basic measurement approaches are
possible: irradiating with monochromatic light simultaneously
or sequentially at a range of angles, or using polychromatic
light at a fixed angle of incidence. The former approach is
usually preferred, especially when irradiation occurs
simultaneously over a range of angles using a wedge-shaped
incident beam. This is described, in relation to SPR, in
European Patent Application No 0305109A.

A disadvantage of known SPR and FTR devices of the kind
described is that unavoidable variations, due to manufacturing

tolerances and the like can mean that the position of the initial resonance varies quite widely from device to device. To accommodate this variation, the angular range of the detector must therefore be much wider than the range over which the position of the resonance shifts during measurements. This increases the cost and complexity of the system, as well as increasing the data processing requirements without concomitant benefit since the majority of the data recorded during each measurement will not contribute to the analysis of the results.

There has now been devised an apparatus involving the use of a resonant optical sensor for the determination of a chemical or biochemical species, which overcomes or substantially mitigates some or all of the disadvantages of the prior art arrangements described above.

According to the invention, there is provided apparatus for the determination of a chemical or biochemical species, comprising a resonant optical sensor disposed in a light path between a polychromatic light source and a detector, there being provided means for irradiating the sensor with light from the light source at a range of angles simultaneously, and the arrangement being such that light reflected from the sensor is allowed to reach the detector only when resonance occurs, and the intensity of the polychromatic light from the light source is modulated as a function of frequency.

The apparatus according to the invention is advantageous primarily in that the intensity modulation applied to the light input to the sensor as a function of frequency is mirrored as a corresponding modulation of the detected light intensity as a function of angle. All of the data accumulated by the detector may then be used to monitor the shift in phase of this modulation which occurs during measurement. The general form of the modulation applied to the input light with frequency is the same, or substantially the same, as the

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modulation of the detected light with angle. If, as is
generally the case, the form of that modulation is known, then
the whole of the detected data may be used in the analysis.
In addition, a smaller detector may be used than in the prior
art. Also, as described more fully below, the components
necessary to provide the polychromatic light and to generate
the modulation are relatively simple, and the form of the
modulation may be chosen at will.

Many different forms of modulation of the input light may be
employed. Periodic modulation is preferred. For ease of
generation and data analysis, a sine wave modulation is
particularly preferred. Where the modulation is between a
maximum value and zero, it it more properly described as a
sine² modulation.

A sine² wave modulation may be generated simply by passing
polarised input light through a so-called phaseplate and a
polariser. In the present case, for light with a wavelength
of the order of 600nm, a sine² intensity modulation with a
period of around 20nm can be produced using a crystal quartz
plate with a thickness of the order of 1mm. Such a multiple
order phaseplate can be readily manufactured and is
sufficiently robust for routine use. The period of the sine
wave modulation may be simply changed by changing the
thickness of the quartz plate.

For typical applications, the bandwidth of the polychromatic
light may be around 200nm, say 800-600nm. Any broad band
light source may be used, eg a tungsten-filament lamp or an
arc lamp.

Simultaneous irradiation over a range of angles is achieved
simply by forming the input beam into a wedge shape, in a
conventional manner. Typically, the range of angles over
which irradiation occurs is 15° or so.

The data recorded by the detector, which is an array of
measured intensities as a function of angle, may be analysed
in various ways. For example, the data may be fitted to a
known function, eg a sine wave-type function. The change in
phase of the wave provides an indication of the extent of
chemical or physico-chemical events at the sensor surface.
Alternatively, the data may be subjected to a Fourier
Transformation, eg using a suitable Fast Fourier Transform
algorithm. Such a transformation provides information not
only about the period of an oscillation, but also its phase.

In a further alternative, a reference sample may be monitored
simultaneously with the sample under investigation and a
comparison made of the results obtained from both. In
particular, the shift in the reference data may be calculated
which is necessary to obtain the best fit with the measured
data for the sample under investigation.

In another embodiment, useful particularly where the period of
the output modulation is small compared with the measured
shift in resonance, there are provided one or more point
detectors at fixed points. These are used to monitor the
number of peaks in the measured signal passing over them as
the output signal shifts during measurement. It is preferred
to use at least two such fixed point detectors, located at
different points on the cycle of the modulated output signal.
This enables shifts of the output signal in opposite
directions to be distinguished.

The sensor may be an FTR sensor. Such a sensor will generally
include an optical structure comprising
a)   a cavity layer of transparent dielectric material of
refractive index n_3,
b)   a dielectric substrate of refractive index n_1, and
c)   interposed between the cavity layer and the substrate, a
dielectric spacer layer of refractive index n_2.

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In use, the interface between the substrate and the spacer layer is irradiated with light such that internal reflection occurs. Resonant propagation of a guided mode in the cavity layer will occur, for a given wavelength, at a particular angle of incidence of the exciting radiation. Where, as in the present invention, polychromatic light is used, resonance for one wavelength will occur at one angle of incidence, and for a different wavelength at a different angle of incidence. Since the intensity of the input radiation is modulated as function of frequency, the intensity of the detected radiation will be modulated in a similar manner as a function of angle.

The angular position of the resonant effect depends on various parameters of the sensor device, such as the refractive indices and thicknesses of the various layers. In general, it is a pre-requisite that the refractive index n_3 of the cavity layer and the refractive index n_1 of the substrate should both exceed the refractive index n_2 of the spacer layer. Also, since at least one mode must exist in the cavity to achieve resonance, the cavity layer must exceed a certain minimum thickness.

The cavity layer is preferably a thin-film of dielectric material. Suitable materials for the cavity layer include zirconium dioxide, titanium dioxide, aluminium oxide and tantalum oxide.

The cavity layer may be prepared by known techniques, eg vacuum evaporation, sputtering, chemical vapour deposition or in-diffusion.

The dielectric spacer layer must have a lower refractive index than both the cavity layer and the substrate. The layer may, for example, comprise an evaporated or sputtered layer of magnesium fluoride. Other suitable materials include lithium fluoride and silicon dioxide. Apart from the evaporation and sputtering techniques mentioned above, the spacer layer may be

deposited on the substrate by a sol-gel process, or be formed
by chemical reaction with the substrate.

The sol-gel process is particularly preferred where the spacer
layer is of silicon dioxide.

The refractive index of the substrate (n_1) must be greater than
that (n_2) of the spacer layer but the thickness of the
substrate is generally not critical.

By contrast, the thickness of the cavity layer must be so
chosen that resonance occurs within an appropriate range of
coupling angles. The spacer layer will typically have a
thickness of the order of several hundred nanometres, say from
about 200nm to 2000nm, more preferably 500 to 1500nm, eg
1000nm. The cavity layer typically has a thickness of a few
tens of nanometres, say 10 to 200nm, more preferably 30 to
150nm, eg 100nm.

It is particularly preferred that the cavity layer has a
thickness of 30 to 150nm and comprises a material selected
from zirconium dioxide, titanium dioxide, tantalum oxide and
aluminium oxide, and the spacer layer has a thickness of 500
to 1500nm and comprises a material selected from magnesium
fluoride, lithium fluoride and silicon dioxide, the choice of
materials being such that the refractive index of the spacer
layer is less than that of the cavity layer.

Preferred materials for the cavity layer and the spacer layer
are tantalum oxide and silicon dioxide respectively.

At resonance, the incident light is coupled into the cavity
layer by FTR, propagates a certain distance along the cavity
layer, and couples back out (also by FTR). The propagation
distance depends on the various device parameters but is
typically of the order of 1 or 2mm. At resonance the
reflected light will undergo a phase change.

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If the sensor is an SPR device it will comprise a dielectric
medium, eg a glass prism, on which is coated a thin layer of
metal. Oscillation of the surface-plasma of free electrons
which exists at the metal-dielectric boundary is affected by
the refractive index of the material adjacent to the metal
surface. For a given wavelength, resonance occurs when the
angle of incidence of the radiation has a particular value,
and this value is dependent on the refractive index of the
material adjacent to the metal. Thus, changes in this
refractive index give rise to to changes in the angle at which
resonance occurs.

A variety of metal coatings may be applied to the glass prism.
Most usually, the metal coating is of silver.

In normal SPR, only TM-polarised (or p-polarised) light is
used, since TE-polarised (or s-polarised) light cannot excite
SPR by the total internal reflection process. Resonance is
detected as a dip in light intensity due to absorbance by the
metal layer at resonance. In the present invention, however,
it is necessary for light to reach the detector at resonance
but not otherwise. This can be achieved by using the
variation on the SPR technique described in International
Patent Application WO 92/04617. In this technique, the
incident radiation contains both TE-polarised and TM-polarised
components and a polarisation analyser is interposed between
the sensor and the detector such that, away from resonance,
little or no light reaches the detector.

For use in the determination of biochemical species, the
surface of the sensor, ie the surface of the cavity layer in
the case of an FTR sensor or the surface of the metal layer in
SPR, will generally be sensitised by having biomolecules, eg
specific binding partners for the analyte(s) under test,
immobilised upon it. The immobilised biochemicals may be
covalently bound to the sensor surface by methods which are
well known to those skilled in the art.

The invention will now be described in more detail, by way of
illustration only, with reference to the accompanying drawings
in which

Figure 1 is a schematic view (not to scale) of an apparatus
according to the invention,

Figure 2 depicts the intensity of the input light as a
function of wavelength, and

Figure 3 depicts the intensity of the detected light as a
function of angle of incidence.

Referring first to Figure 1, an FTR biosensor comprises a
glass prism 1 coated over an area of its base with a first
coating 2 of magnesium fluoride and a second coating 3 of
titanium dioxide. The prism 1 and first and second
coatings 2,3 together constitute a resonant optical structure,
the first coating 2 acting as a spacer layer and the second
coating 3 as a cavity layer. The first coating 2 has a
thickness of approximately 1000nm and the second coating 3 a
thickness of approximately 100nm.

Immobilised on the surface of the second coating 3 is a
layer 4 of immobilised biochemicals, which act as specific
binding partner for the analyte under test.

The interface between the base of the prism 1 and the first
coating 2 is irradiated by a beam of polychromatic light from
a tungsten-filament lamp 5. Light from the lamp 5 is
collimated by a lens 6 and then passes through a first
polariser 7, a phaseplate 8 and a second polariser 9. The
light is then formed into a wedge-shaped input beam by a
focussing lens 10. The sensor is thus irradiated with
polychromatic light with a range of angles of incidence
simultaneously. The range of angles is approximately 15°.

The first polariser 7 and the second polarizer 9 are arranged
at 45° to the plane of incidence. Light incident on the
biosensor is therefore linearly polarised with two equal
components : transverse electric (TE) and transverse magnetic
(TM).

The phaseplate 8 is a sheet of quartz approximately 1mm in
thickness. The phaseplate 8 is oriented with its
crystallographic axes at 45° to the plane of polarisation of
the first polarizer 7 and the second polarizer 9. The effect
of the phaseplate 8 and the second polarizers 9 is to modulate
the intensity of the input light as a function of frequency.
A sine^2 wave modulation is applied to the frequency spectrum of
the input light, as shown in Figure 2. The modulation has a
period of about 20nm.

All the light incident on the interface between the base of
the prism 1 and the first coating 2 is reflected. For each
angle of incidence, resonance occurs for a incident radiation
of a particular wavelength.

Resonance is detected as a change of phase of the reflected
light. The reflected light passes through a compensator 12 to
a polarisation analyser 13. The compensator 12, which may be
of any conventional form, is manually adjusted to remove any
phase difference which is introduced into the TE and TM
components on reflection and by birefringence in the optical
path.

The analyser 13 is arranged at 90° to the first and second
polarisers 7,9. The TE and TM components are interfered at
the analyser to allow the phase change to be detected. Off
resonance both components undergo a similar phase shift on
reflection and the relative phase between the components is
adjusted by the compensator 12 to give zero transmission
through the analyser 13. This will apply for all angles
except near resonance. Near resonance of either component,

the phase shift between the TE and TM components will vary
rapidly with angle, resulting in maximum throughput of light
through the analyser 13 at resonance.

Light passing through the analyser 13 is detected by a
detector 15 comprising an array of charge coupled devices
(CCD).

The measured light intensity is modulated as a function of
angle in the same way as the input light intensity is
modulated as a function of wavelength, as shown in Figure 3.

When the layer of immobilised biochemicals 4 is contacted with
a sample containing the analyte under test, specific binding
occurs between the biochemicals and the analyte molecules,
resulting in a change in the refractive index in the vicinity
of the surface of the device. This in turn results in a shift
in the position of the resonance for each incident wavelength.
Figure 3 shows a plot of the measured signal intensity against
angle of incidence before and (dotted line) after complexation
of the immobilised biochemicals with the analyte. As can be
seen, the whole measured signal is shifted one way or the
other by the complexation reaction.

The measured signal may be analysed in several ways. For
instance, the signal may be fitted using conventional fitting
algorithms to a sine-wave function (or a modified sine-wave
function to take account of any distortions which occur).
Alternatively, the signal may be subjected to a Fourier
transformation. In any event, all of the accumulated data
points are used to assess the extent to which the signal is
shifted left or right.

<u>Claims</u>

1.   Apparatus for the determination of a chemical or
biochemical species, comprising a resonant optical sensor
disposed in a light path between a polychromatic light source
and a detector, there being provided means for irradiating the
sensor with light from the light source at a range of angles
simultaneously, and the arrangement being such that light
reflected from the sensor is allowed to reach the detector
only when resonance occurs, and the intensity of the
polychromatic light from the light source is modulated as a
function of frequency.

2.   Apparatus as claimed in Claim 1, wherein a periodic
modulation is applied to the polychromatic light.

3.   Apparatus as claimed in Claim 2, wherein the modulation
is a $sine^2$ modulation.

4.   Apparatus as claimed in Claim 3, wherein the $sine^2$
modulation is generated by passing polarised input light
through a phaseplate and a polariser.

5.   Apparatus as claimed in Claim 4, wherein the phase plate
is a crystal quartz plate with a thickness of the order of
1mm.

6.   Apparatus as claimed in any preceding claim, wherein the
sensor is an FTR sensor including an optical structure
comprising
a)   a cavity layer of transparent dielectric material of
refractive index n_3,
b)   a dielectric substrate of refractive index n_1, and
c)   interposed between the cavity layer and the substrate, a
dielectric spacer layer of refractive index n_2.

7.   Apparatus as claimed in Claim 6, wherein the cavity layer

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is a thin film of dielectric material.

8.   Apparatus as claimed in Claim 7, wherein the spacer layer
has a thickness of from about 200nm to 2000nm, and the cavity
layer has a thickness of 10 to 200nm.

9.   Apparatus as claimed in any one of Claims 1 to 5, wherein
the sensor is an SPR device comprising a dielectric medium on
which is coated a thin layer of metal.

10. Apparatus as claimed in Claim 9, wherein the metal is
silver.

11. Apparatus as claimed in Claim 9 or Claim 10, wherein the
incident radiation contains both TE-polarised and TM-polarised
components and a polarisation analyser is interposed between
the sensor and the detector such that, away from resonance,
little or no light reaches the detector.

12. Apparatus as claimed in any preceding claim, wherein the
sensor is a biosensor for use in the determination of
biochemical species, and a surface of the sensor is sensitised
by having biomolecules immobilised upon it.

13.   A method of determining a chemical or biochemical
species in a sample, which method comprises the steps of
a)   irradiating a resonant optical sensor with polychromatic
light from a light source at a range of angles simultaneously,
b)   applying to the incident light a modulation as a function
of frequency,
c)   permitting light reflected from the sensor to reach a
detector only when resonance occurs,
d)   contacting the sample with the sensor, and
e)   monitoring the intensity of the reflected light reaching
the detector as a function of the angle of incidence.

14. A method as claimed in Claim 13, comprising the further

step of fitting the measured intensities to a known function.

15.   A method as claimed in Claim 13, comprising the further
step of subjecting the measured intensities to a Fourier
Transformation.
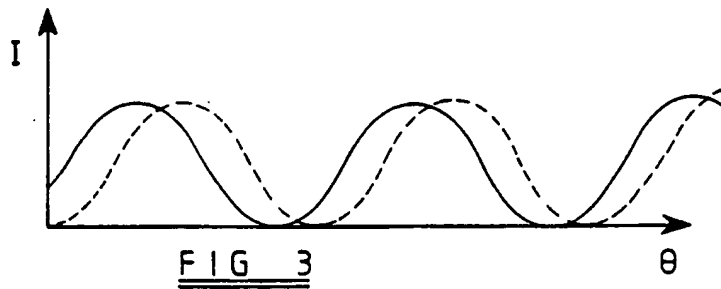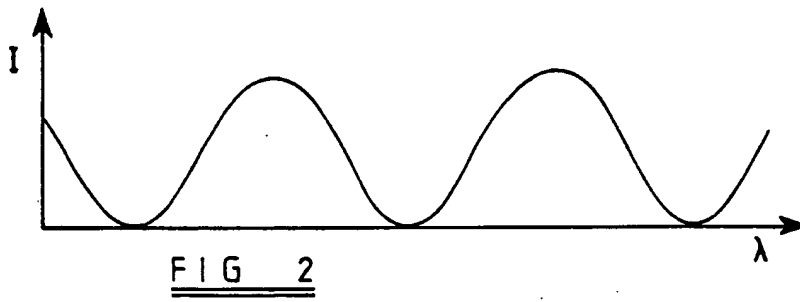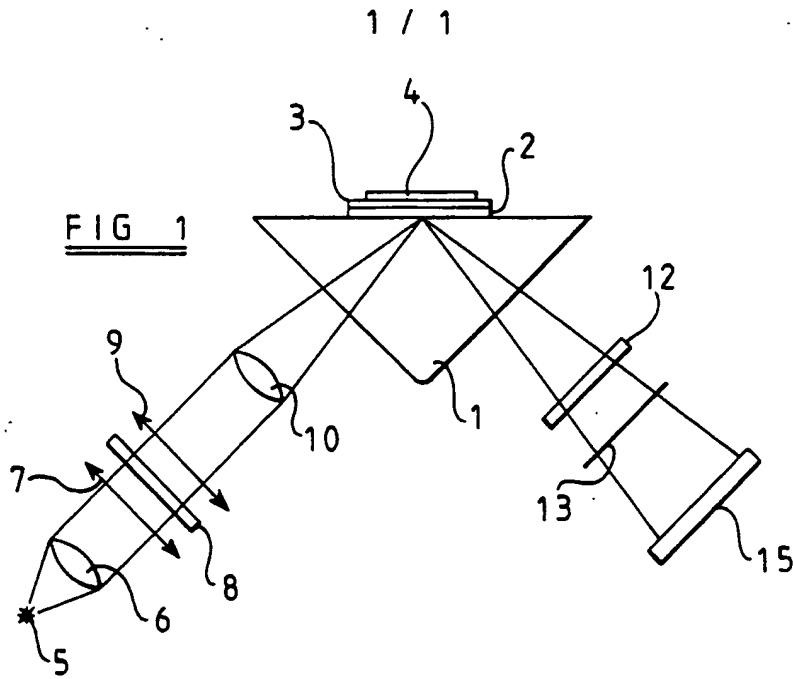
FIG 1

FIG 2

FIG 3

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5   G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5   G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 04617 (FISONS) 19 March 1992 cited in the application see page 2, paragraph 3 see page 5, paragraph 3 see page 7, paragraph 5 - page 8, paragraph 2 see claims 1,2,7,8,11; figures 1,2 ---	1,9-13
A	WO,A,92 03720 (FISONS) 5 March 1992 see page 5, paragraph 3 - page 7, paragraph 2 see page 8, paragraph 2 see page 10, paragraph 2 -----	1,6-8, 12,13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9204617	19-03-92	EP-A- 0546061	16-06-93
WO-A-9203720	05-03-92	EP-A- 0543831	02-06-93
		JP-T- 5509402	22-12-93